Center for Veterinary Biologics
and
National Veterinary Services Laboratories
Testing Protocol

Supplemental Assay Method for the Manual Determination of Protein Content of Veterinary Biologics (Biuret)

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Supplemental Assay Method for the Manual Determination of Protein Content in Veterinary Biologics (Biuret)

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1. Introduction

The measurement of the protein content of various veterinary biologics products (serum, antiserum, and antitoxins) is often utilized in the evaluation of such products. The following details the classical biuret procedure for the indirect determination of protein concentration (**References 7.1, 7.2, 7.3, 7.4**).

2. Materials

2.1 Equipment/instrumentation

2.1.1 Spectrophotometer or colorimeter--Bausch and Lomb Spectronic 70 with cuvettes (or colorimeter with 1 cm or greater path length)

2.1.2 Common laboratory apparatus and glassware-- pipets, pipetors with tips, screw cap tubes, class A volumetric flasks, linear graph paper

2.1.3 Computer--with linear regression program (optional)

2.2 Reagents/supplies

2.2.1 Phosphate buffered saline (PBS)--0.01M, pH 7.2-7.4 (**NVSL media 30033**), store at 4 C, stable for at least 6 months.

2.2.2 Biuret reagent--(**NVSL media 10307**), store at room temperature, stable for at least 6 months.

Critical control point: Biuret reagent should be replaced when crystals or other precipitates appear in the solution.

2.2.3 Standard protein solution--Crystalline bovine albumin containing a known amount of protein.

2.2.4 Bovine serum reference--Normal bovine serum [**NVSL media 40032**, bovine serum (donor)], store at -20 C, stable at least 1 year.

3. Preparation for the test

3.1 Personnel training--No specific training is required. Individuals should have working knowledge of laboratory equipment listed in **Section 2**.

3.2 Equipment--Turn on spectrophotometer to allow instrument to "warm up" for at least 30 minutes.

3.3 Reagents--Prepared by the NVSL media section.

3.4 Sample--Samples are normally sera, antisera or antitoxins, or serum fractions. Occasionally, biuret tests are run on other solutions, such as antigens. Reference the latest version of **TCSOP0001** for sample submission.

4. Performance of test

4.1 Standards

4.1.1 Standard solutions--Dilute Bovine Albumin with PBS to contain 10 mg/ml protein. Use 10 mg/ml solution as stock for curve. Dilute as listed below to establish a working standard curve.

CONC.(mg/ml)	ML STOCK	ML PBS
10	1.0	0
8	0.8	0.2
6	0.6	0.4
4	0.4	0.6
2	0.2	0.8
1	0.1	0.9

4.1.2 Run duplicate tubes of each solution (**4.1.1**) using the test method (**4.2**) to establish a standard curve. Plot average optical density (OD) for each point on graph paper (concentration vs OD) or enter data into computer program to plot curve and calculate test results. If OD values for any point differ more than 0.05, disregard that point. If more than one point has unacceptable OD variations, rerun standard curve.

Critical control point: A standard curve is accurate for that lot of Biuret reagent. A comparison run or a new curve must be run when a new lot is used.

4.2 Test method

4.2.1 Dilute sample and bovine serum control 1:10 or 1:20 with PBS. Mix gently. (Sample dilution is based on the sample appearance or prior knowledge of sample. Dilute sample so OD falls on standard curve.)

4.2.2 Transfer 1 ml of dilution (**4.2.1**) to tube or cuvette. Run duplicates.

4.2.3 Transfer 1 ml PBS to tube or cuvette for instrument blank.

4.2.4 Add 4 ml biuret reagent to each tube and mix gently. Let stand at room temperature for 30 - 45 minutes for color development.

4.2.5 Read OD at 540 nm, using blank to set zero. Read and record OD.

5. Interpretation of results

5.1 Calculation--Determine sample value (either read from curve and multiply x dilution or enter data into computer program). Average test results of duplicate tests. Test results are acceptable if the duplicate test results vary no more than 5% from the mean and the protein value for the bovine serum control falls within 5% of the established value.

5.2 Retest--If the OD of the diluted sample reads outside the end points on the standard curve, redilute sample and rerun the test.

6. Report test results

6.1 Test results are reported following the current version of **TCSOP0001.**

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7. References

7.1 Robinson, H. W. and Hogden, C. G. (1940) J. Biol. Chem., vol. 135, 707-725.

7.2 Gornall, A. G.,Bardawill, C. J., and David, M. M. (1949) J. Biol. Chem., vol. 177, 751-766.

7.3 Kibrick, A.C. (1958) Clin. Chem., vol. 4, 232-236.

7.4 Kingsley, G. R. (1939) J. Biol. Chem., vol. 131, 1971.

7.5 Reference is made to the long term use of this procedure by the Toxicology and Chemistry Section.

7.6 This protocol is a revision of SAM 504, September 1, 1970, to the current format. Version .02 was generated to reflect the creation of the Center for Veterinary Biologics. Neither version .01 nor .02 contain technical changes from the original SAM 504.

Appendix 8.1--NVSL Media 30033

Media no.: 30033, PBS 0.01 M (Sterility Section, TC Section)

Formula:	Quantity per liter
potassium phosphate, monobasic	0.34 g
sodium phosphate dibasic, anhydrous	1.10 g
sodium chloride	8.50 g
sodium phosphate, dibasic	0.15 g

pH: 7.2-7.4

Container: 1 liter glass bottle

Method of sterilization: autoclave

Special instructions: Dissolve ingredients in 400-500 ml water and dilute to volume.

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Appendix 8.2--NVSL Media 10307

Media no.: 30033, Biuret Reagent

Formula:	Quantity per liter
copper sulfate, pentahydrate	1.50 g (dissolve in 400 ml water)
potassium sodium tartrate	6.00 g (add to above and dissolve)
sodium hydroxide	30.0 g (dissolve in separate 300 ml water, add to above)
potassium iodide	1.00 g (add to above after other chemicals are dissolved and dilute to 1 L)

pH: not applicable

Container: 1 liter glass bottle

Method of sterilization: none

Special instructions: none

Appendix 8.3--NVSL Media 40032

Media no.: 40032, Bovine serum--not sterilized

Container: 5 ml serum vial

Method of sterilization: none

Special instructions: Donor bovine serum, mix thoroughly, dispense 3 ml into serum vials

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9. Quick Reference

_____Accession number and section number assigned

_____Submission paperwork correct

_____Adequate amount of sample(s)

_____Buiret (date prepared)

_____Sample(s) OD within standard curve limits

_____Results reviewed

_____Report generated

_____Report reviewed, signed, and sent